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# Virus structures: **Those magnificent molecular machines**

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**The core particle of a double-stranded RNA virus acts as a complex RNA replication machine. The recently solved crystal structure of the reovirus core particle has yielded a wealth of information, from the conserved architecture of double-stranded RNA viruses to a description of the mRNA capping machinery.**

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**Current Biology** 2000, 10:R558–R561

0960-9822/00/$ – see front matter
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Double-stranded RNA viruses face two major problems: the endogenous machinery of its host cells does not replicate RNA genomes, and double-stranded RNA provokes strong cellular defence responses such as interferon induction and gene silencing. The solution for the virus is to keep the genomic double-stranded RNA always in a separate container, an icosahedral viral capsid, and to more-or-less autonomously replicate the genome with machinery built into this capsid. In most Reoviridae, this internal capsid structure is surrounded by two additional protein layers that are used in receptor recognition and cell entry.

The replication cycle of a segmented double-stranded RNA virus genome is depicted schematically in Figure 1. The transcription reaction, which leads to plus-strand synthesis, and the RNA capping reaction have been well documented for many members of the Reoviridae. But the genome packaging mechanism, and consequently the site of minus-strand synthesis, has not been well defined. This part of the replication cycle has, however, been well documented in the case of the bacterial double-stranded RNA virus phi6 [1]. I shall take the liberty of using phi6 as a model case to illustrate the entire replication cycle of a double-stranded RNA virus. By replication, I refer to the duplication of the parental double-stranded RNA to form the progeny double-stranded RNA genome (often minus-strand synthesis alone is referred to as replication).

The following enzymatic activities, summarized in Figure 1, are associated with the internal capsid or core particle. The entering core is activated to synthesize mRNAs using all the double-stranded RNA genome segments as templates — up to 12, depending on the particular virus. These mRNAs exit the particle, and in the case of eukaryotic double-stranded RNA viruses, the 5′ end of each mRNA molecule is capped upon exit by the viral capping system located around each of the icosahedral five-fold axes, which function as single-stranded RNA exit sites. The mRNAs are used either to direct protein synthesis for the assembly of new polymerase particles or as genomic precursors that are packaged into the newly formed empty precursor particles. This RNA translocation and condensation event requires mechanical energy, obtained from nucleoside triphosphate hydrolysis catalysed out by a viral packaging enzyme which is also located at the icosahedral five-fold axes of the capsid.

Once inside the particle, the single-stranded RNA segments are converted to double-stranded molecules by the same enzyme that carries out transcription. It is most probable that there is one copy of the polymerase subunit close to each of the five-fold axes — twelve in total. Although this description combines information gained from studying different double-stranded RNA viruses, it is clear that these viral cores are molecular machines with a number of fundamental enzymatic activities and the capability to select, package and replicate up to twelve genome segments with high fidelity. When one adds that the packaging density of the genome inside the particle — about 400 mg/ml — is so high that it leads to a liquid crystalline state [2], one appreciates the order and functionality of this viral polymerase particle.

Reinisch *et al.* [3] have now reported the structure of the reovirus core particle at 3.6 Å resolution, a remarkable achievement. The particle mass is about 52,000 kDa and its diameter some 700 Å. It is composed of more than 350 proteins and 10 segments of double-stranded RNA. The crystal growth took about a year and tiny (150 μm) fragile crystals appeared that did not withstand cryopreservation. The unit cell — the fundamental repeat unit within the crystal — is huge. More than 500 diffraction patterns were included in the data set and many more were collected. A project of this kind needs advanced science and technology and a lot of tenacity. The structure was solved with the help of a 27 Å resolution three-dimensional reconstruction of the core, which had been determined earlier by cryoelectron microscopy [4].

What is seen in the solved structure? The core particle is composed of five different proteins. Three of these proteins — λ1, present in 120 copies, λ2 in 60 copies and σ2 in 150 copies — are symmetrically arranged, and most of their amino-acid residues are visible in the refined structure. The other two proteins, the polymerase λ3 and a minor core protein μ2, are not visible because of their

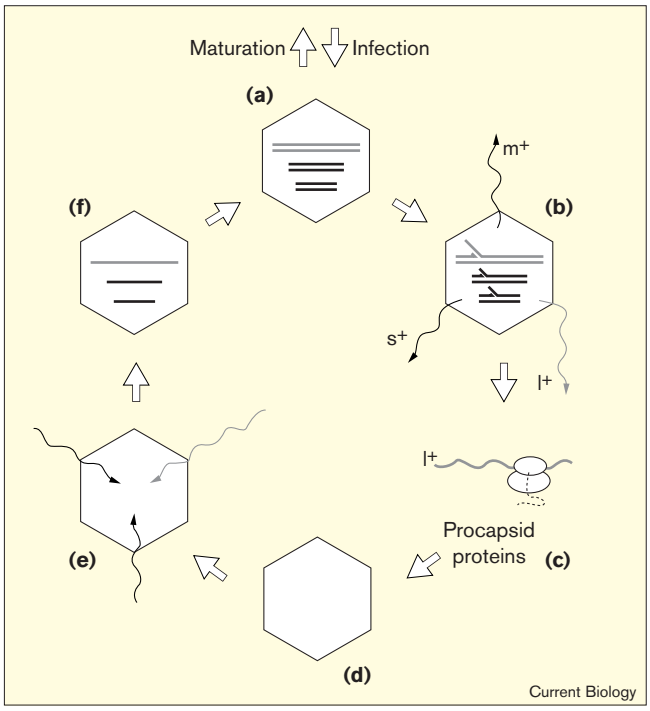
monomeric state. The particle shell is made of the 120 λ1 subunits, an arrangement that is unusual in icosahedral virus capsid arrangements, where the number of subunits, *T*, that occupy an icosahedral asymmetry unit — 1/60th of the icosahedron — follows the formula $T = h^2 + hk + k^2$, where *h* and *k* are integers. *T* numbers such as 1, 3, 4, 7, and so on, thus determine the capsid architecture and the number of different contacts made by the subunits. The arrangement in the reovirus core can be viewed as two non-equivalent λ1 proteins, referred to as λ1A and λ1B, making the asymmetry unit. The protein contains two reasonably rigid subdomains and a shift between allows conformational switching. This arrangement has been described in the case of bluetongue virus — another member of Reoviridae — as geometric quasi-equivalence and referred to as $T = 2$ ([5], see below).

In the case of reovirus, it appears that no icosahedral particles are formed in the cell if the λ1 protein is not accompanied by σ2 [6]. This globular monomeric protein occupies three distinct locations within each icosahedral asymmetry unit. Two of them are completely unrelated, one associated with λ1A and one with λ1B. At the third site, σ2 has two opposite orientations at an icosahedral dyad symmetry axis. The two unique binding sites are of high affinity and it is evident that this protein can interact specifically and stably in several unrelated ways. This adds another dimension to the concept of specific biological interactions as one protein can, with minor conformational modifications, interact with very different targets. The reovirus core assembly may depend on the recognition and stabilizing of the λ1 interphases by σ2.

The reovirus core structure [3] is the first high resolution structure of a double-stranded RNA virus that sheds light on any of the enzymatic activities depicted in Figure 1. Around the five-fold symmetry axis on the top of the λ1A pentamer resides a hollow turret made of five λ2 subunits. This turret is about 80 Å high and 120 Å in diameter and is responsible for capping the 5′ end of nascent mRNA. The central cavity is wide at the bottom, but narrows to an opening that can accommodate a single-stranded RNA molecule.

The λ2 monomer is a seven domain protein. The amino-terminal domain makes the contacts with the $T = 2$ shell and has two critical lysine residues facing the cavity; these lysines are crucial for the guanylyltransferase capping activity [7]. There are two methyl transferase domains further up the λ2 structure: one is responsible for the *N*7 methylation and the other for *2′O* methylation. The active methylation sites are formed by two adjacent monomers explaining the lack of methylase activity in the monomer. As the *N*7 methylation precedes the *2′O* methylation, Reinisch *et al.* [3] suggest that the proximal methylation site is responsible for the *N*7 and the distal one for the *2′O* methylation. Figure 2 illustrates the reovirus core structure and the proposed localization of polymerase as well as the exit site of the capped mRNA.

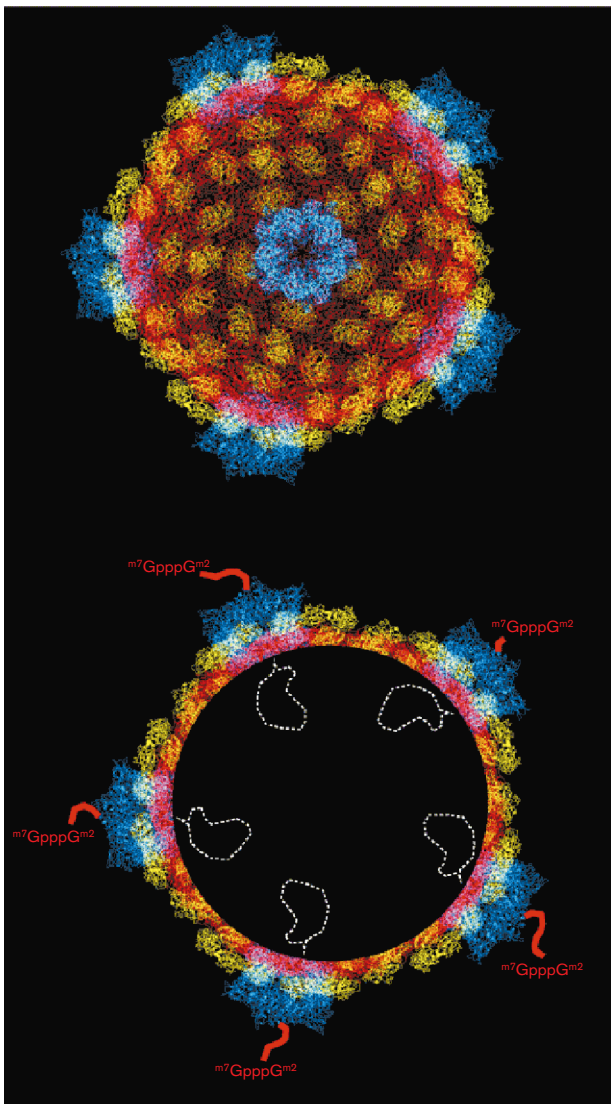
**Figure 1**




The genome replication cycle of a double-stranded RNA virus (see the text). The infection process brings into the cell the core containing the double-stranded RNA genome segments **(a)**. Upon uncoating, the particle is activated to synthesize single-stranded RNA copies from each genome segment; these exit through a machinery at the icosahedral vertices **(b)**. The single-stranded RNA segments are of positive polarity and are used as mRNAs **(c)**, leading the synthesis of proteins which assemble to new polymerase complex particles **(d)**. The transcripts are also used as genomic precursors that are packaged into the procapsids also through the machinery at the icosahedral vertices **(e,f)**. This process requires energy in the from of NTPs. The packaged single-stranded RNA genomic precursors are used as templates to produce progeny genomic double-stranded RNA segments by the polymerase subunit associated within the particle (f,a). The packaged particles mature into infectious virions that are released from the cells.

The peculiar $T = 2$ structure, with 120 copies of the capsid protein, was first observed in the case of the bluetongue virus core particle [5]. From a comparison of the high resolution structures with biochemical data and information obtained from lower-resolution electron microscopic reconstructions, all of the double-stranded RNA viruses that have been studied have this $T = 2$ arrangement; this arrangement has not been yet observed in any other type of virus. These viruses also share the same basic principles of RNA metabolism. The ‘business end’ is in all cases associated with the five-fold symmetry axis, where the proteins responsible for the enzymatic activities are located (the fungal double-stranded RNA viruses are

**Figure 2**




The reovirus core structure (top) and a schematic representation of the core organization (bottom). The different types of subunit are highlighted in different colours: λ1 in red, σ2 in yellow and the λ2 pentamers in blue. A polymerase complex (white), embedded in concentric layers of double-stranded RNA, is believed to sit below each of the turrets so as to pass the single-stranded RNA (red) out of the particle and through the capping complex. As the 5′ RNA terminus passes through the turret the capping reactions take place in an orderly fashion (see the text). Adapted with permission from [3].

somewhat simplified versions of this theme, which probably reflects their nature as intra-cellular parasites only).

The Reoviridae can be devided into two distinct classes. One, represented by reovirus, consists of the viruses that have extensive turrets and either a loosely-associated protein shell around the $T = 2$ capsid or no such protein layer at all [8]. The other class is exemplified by bluetongue virus; these viruses have a well ordered, $T = 13$, protein shell around the polymerase complex and no turrets but an elaborate structure at the five-fold axis that extends inwards from the particle surface. Interestingly, the bacterial double-stranded RNA virus phi6 has both a rigid $T = 13$ layer and turrets [9]. Although it is not obvious how to quantitatively approach the probabilities that all these common properties have occurred just by chance, one cannot escape the obvious suggestion that the double-stranded RNA viruses share common ancestry.

What next? Because of symmetry constraints the monomeric polymerase protein resists structural analysis in connection with the particles. To gain structural information on how linear single-stranded and double-stranded RNA molecules are used as templates awaits determination of high-resolution structures of the individual polymerase subunit. This project is well underway with both phage phi6 and reovirus polymerases. How to tackle the selective packaging of the up to twelve individual genome segments is also an intriguing question. Is it possible that a high number of genome segments are selectively co-condensed with the assembling $T = 2$ shell. I personally favour the phi6 mechanism, where the preformed shell distinguishes between the segments to guarantee equimolar packaging [1,9,10]. This is analogous to other complex viruses, where a pathway is used in which a procapsid is the precursor for packaging and maturation [11].

**Acknowledgements**

Eugeny Makeyev is thanked for providing Figure 1 and Karin Reinisch for Figure 2. Sarah Butcher is thanked for critical reading of the manuscript. This effort was supported by Finnish Center of Excellence Programme (2000-2005) of The Academy of Finland.

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